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Determination of an Inoculum Effect with Various Cephalosporins among Clinical Isolates of Methicillin-Susceptible *Staphylococcus aureus*[▽]

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Using 98 clinical methicillin-susceptible *Staphylococcus aureus* isolates of known β-lactamase (Bla) type, we found a pronounced inoculum effect for cephalexin (mostly Bla type A and C strains), a mild inoculum effect for cephalothin (especially types B and C), and no inoculum effects for ceftriaxone and cefuroxime. Ceftobiprole showed the lowest MICs at a high inoculum but with a slight increase for Bla-positive versus Bla-negative strains. Since a potential therapeutic effect associated with a cephalosporin inoculum effect has been described, further studies are warranted.

Strains of methicillin-susceptible *Staphylococcus aureus* (MSSA) are still responsible for the majority of severe staphylococcal infections, as shown in a recent international study in which 85.2% of *S. aureus* strains producing native valve endocarditis were susceptible to methicillin (11). About 90% of the MSSA isolates produce one of four variants of β-lactamase(s) (Bla) (16) identified as type A, B, C, or D (9, 13, 22). Each of these staphylococcal Bla types has a specific substrate profile (21). These severe MSSA infections are often treated with cephalosporins, and even though cephalosporins are regarded as generally stable in the presence of staphylococcal Bla, it was shown early on that when tested at higher inocula, some cephalosporins were hydrolyzed by certain types of Bla (inoculum effect) (10). It was not until the 1990s, when the kinetics of hydrolysis among the four types of staphylococcal Bla were reported, that the classification of cephalosporins as Bla stable or labile was considered an oversimplification of the cephalosporin-Bla interaction (23). However, the actual effect of each type of staphylococcal Bla on the *in vitro* activity of different cephalosporins, measured by the presence of an inoculum effect, has not been clearly defined. Strains producing a large amount of the Bla enzyme and/or showing high MICs when a large inoculum is used have been associated with clinical failures in patients suffering high-inoculum staphylococcal disease (e.g., cefazolin in the treatment of endocarditis caused by MSSA producing type A Bla) (2, 4, 12, 15). We have recently shown that about 20% of MSSA clinical strains displayed an elevated cefazolin MIC (≥16 μg/ml) when tested at a high inoculum (13). Here, using high-inoculum MIC determinations, we determined the presence of an inoculum effect for several cephalosporins by using a set of clinical MSSA isolates previously classified by the type of Bla produced (13).

Ninety-eight clinical MSSA strains that have been previously reported were included in this analysis, as follows: 25 type A, 15 type B, 45 type C, and 0 type D Bla strains and 13 *blaZ*-negative strains (13). *S. aureus* strain TX0117, a high-level producer of type A Bla (12); *S. aureus* ATCC 29213, known to produce small amounts of type A Bla (9); and *S. aureus* ATCC 25923, a Bla-negative strain, were used as controls. MICs were determined by the broth microdilution method, using the Clinical and Laboratory Standards Institute (CLSI) guidelines (3). MICs were determined using standard (~5×10^5 CFU/ml) and high (~5×10^7 CFU/ml) inocula for all the tested antibiotics except cefuroxime (tested only at the high inoculum) and were read at 24 h. Inocula were estimated by optical density (OD) with random determination of CFU/ml. Ceftriaxone, cefuroxime, cephalexin, cephalothin, and nafcillin were obtained from Sigma Chemicals (Sigma, St. Louis, MO). Ceftobiprole was provided by Johnson & Johnson Pharmaceuticals. The MIC_{90}s and geometric mean MICs were calculated, and the comparisons between the geometric mean MICs of each antibiotic among the different types of Bla-producing strains and between the Bla-positive and Bla-negative ones were performed with the nonparametric Mann-Whitney test. For all calculations, a two-tailed P value of <0.05 was considered to be statistically significant. All statistical comparisons were performed using the NCSS/PASS Dawson Edition program (Kaysville). This study was reviewed and approved by the Duke University Institutional Review Board.

The MICs at the high inoculum and standard inoculum of the

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TABLE 1. MIC_{90}s, geometric mean MICs, and range of MICs of nafcillin and several cephalosporins at a high and standard inoculum among Bla-producing and non-Bla-producing MSSA strains[c]

Agent	Inoculum	Value (μg/ml) for:			
		Bla-positive strains (n = 85)		Bla-negative strains (n = 13)	
		GM MIC	MIC_{90}	GM MIC	MIC_{90}
Nafcillin	SI	0.4	0.5	0.4	0.5
	HI	0.7[a]	1	0.7[a]	1
Cephalexin	SI	3.9	4	2.5	4
	HI	10.7[b]	32	3.1[b]	4
Cephalothin	SI	0.5	1	0.3	0.5
	HI	1.9[b]	8	0.3[b]	0.5
Ceftobiprole	SI	0.4	0.5	0.3	0.5
	HI	0.7[b]	1	0.3[b]	0.5
Ceftriaxone	SI	2.9	4	3.1	4
	HI	3.4[a]	4	3.1[a]	4
Cefuroxime	HI	1[a]	1	1[a]	1

[a] The P value for nafcillin, ceftriaxone, and cefuroxime is >0.05. The P value is for the GM MIC of Bla-positive strains versus the GM MIC of Bla-negative strains.

[b] The P value for cephalexin, cephalothin, and ceftobiprole is <0.0001. The P value is for the GM MIC of Bla-positive strains versus the GM MIC of Bla-negative strains.

[c] GM, geometric mean; SI, standard inoculum (~5×10^5 CFU/ml); HI, high inoculum (~5×10^7 CFU/ml).

TABLE 2. Geometric mean MICs and MIC_{90}s of cephalexin, cephalothin, and ceftobiprole at high and standard inocula according to the type of Bla produced among MSSA strains[d]

Agent	Inoculum	Value (μg/ml) for indicated type Bla producers:					
		A (n = 25)		B (n = 15)		C (n = 45)	
		GM MIC	MIC_{90}	GM MIC	MIC_{90}	GM MIC	MIC_{90}
Cephalexin	SI	4.6	8	3.3	4	3.8	4
	HI	14.7[a,b]	64	6.6[a,c]	16	10.6[b,c]	32
Cephalothin	SI	0.4	0.5	0.5	1	0.6	1
	HI	1.3[a,b]	4	3[a,c]	8	2[b,c]	8
Ceftobiprole	SI	0.4	0.5	0.5	0.5	0.5	0.5
	HI	0.7[a,b]	2	0.7[a,c]	1	0.7[b,c]	1

[a] For type A versus type B: cephalexin, P = 0.014; cephalothin, P = 0.015; ceftobiprole, P value is not significant (NS).

[b] For type A versus type C: cephalexin, P = NS; cephalothin, P = 0.015; ceftobiprole, P = NS.

[c] For type B versus type C: cephalexin, P = 0.018; cephalothin, P = NS; ceftobiprole, P = NS.

[d] GM, geometric mean; SI, standard inoculum (~5×10^5 CFU/ml); HI, high inoculum (~5×10^7 CFU/ml).

five cephalosporins and nafcillin for the Bla-positive and Bla-negative isolates are displayed in Table 1. Table 2 describes the MICs of cephalexin, cephalothin, and ceftobiprole at the high and standard inocula according to the type of Bla produced.

A significant increase in the MICs of cephalexin was observed when Bla-positive strains were compared with Bla-negative ones. As a measure of the degree of the inoculum effect, 36.7% of the MSSA strains displayed a high-inoculum MIC of cephalexin at above the CLSI breakpoint for nonsusceptibility at the standard inoculum (≥16 μg/ml). This inoculum effect was seen with all three types of Bla-producing strains studied, although type A and type C strains displayed higher MICs than did type B strains. The clinical implications of these findings are unclear since cephalexin is frequently used to treat MSSA infections where either a high inoculum is not present or the infection is drained. The cephalothin MICs were modestly but significantly higher for the Bla-producing strains versus the Bla-negative group. An increase in the high-inoculum cephalothin MICs (8 μg/ml) was observed in 13% of the strains, most of which were type B and C Bla-producing strains. The difference observed in the geometric mean MICs between type B and type C strains versus type A ones was statistically significant. Some degree of inoculum effect for cephalothin by the use of untyped Bla-producing isolates has been previously described (17), and higher hydrolysis rates were observed among type B and C Bla than those for types A and D by Zygmunt et al. (23). In addition, lower efficacy of cephalothin was reported in an endocarditis model with an untyped Bla-positive MSSA strain with a high-inoculum cephalothin MIC (7).

Ceftriaxone showed the second highest geometric mean MIC at the standard inoculum. However, the activity of ceftriaxone was not affected by the inoculum size; at the high inoculum, one type C Bla-producing strain had a ceftriaxone MIC of 32 μg/ml, and a Bla-negative one had a ceftriaxone MIC of 16 μg/ml. Ceftriaxone was as effective as cloxacillin in the treatment of MSSA experimental endocarditis in rabbits (6) and has been used to treat a small series of patients with MSSA endocarditis (5) and osteomyelitis (8).

Cefuroxime was tested only at the high inoculum but did not appear to be affected by the production of Bla since the geometric mean MICs at the high inoculum of Bla-producing strains was similar to that seen among the Bla-negative strains. The relative stabilities of cefuroxime have been previously described, although using few Bla-positive strains of unknown type (18). Cefuroxime has shown similar efficacies to methicillin and cefazolin in the treatment of experimental MSSA endocarditis (19) and appeared to have greater efficacy than cefazolin in an endocarditis model using an MSSA strain with high-level production of type A Bla (20).

Ceftobiprole, recently approved for clinical use in Canada and Switzerland and advocated as a potent compound for both MSSA and methicillin-resistant *S. aureus* (MRSA) isolates, has been previously described as a poor substrate for type A Bla (14). Here, ceftobiprole showed the lowest high- and standard-inoculum MICs among the four types of Bla-positive strains. However, a slight but significant difference was observed between the high-inoculum geometric mean MICs of the Bla-positive strains and those of Bla-negative strains, suggesting that some hydrolysis occurs.

The presence of an inoculum effect indicates a need for an increased concentration of the tested antibiotic to achieve growth inhibition in the presence of higher numbers of bacterial cells. However, the inoculum effect is a dynamic process where other factors are involved. For instance, a marked inoculum effect might not be significant in a drained infection

and/or when high antibiotic concentrations are achieved at the infection site. On the other hand, a mild inoculum effect might be significant in an infection with a high number of bacterial cells and/or with low concentrations of the antibiotic at the specific infection site. Considering that up to 50% of the staphylococcal Bla is excreted from the bacterial cells (1), the concentration of this enzyme at the infection site could be considerable in high-inoculum infections. In this situation, if the infecting MSSA strain produces large amounts of an active Bla, the enzyme may inactivate its target at a rate high enough to overcome its antibacterial effect. An association between cefazolin and clinical failures in patients with endocarditis (2, 4, 12, 15) caused by MSSA strains displaying an inoculum effect with cefazolin appeared to be well documented (12, 15). In this study, we expand the knowledge about the effects of the different types of Bla measured by the inoculum effect on the tested cephalosporins. Further studies are required to determine if the presence of an *in vitro* inoculum effect to certain cephalosporin might affect the clinical therapeutic response in serious MSSA infections.

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